HUMAN COLLAGEN PROCESSING AND AUTOIMPLANT USE

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to human collagen processing and autoimplant use, and more particularly to a chemically modified, crosslinkable, telopeptide-containing, naturally crosslinked, solubilized collagenous substance obtained directly from intact human tissue from a sole human donor, for implanting in various forms in the same said donor, and to the process for making such product.

In general, the collagens are ubiquitous proteins found throughout the animal kingdom. All known collagens are rod-like structures. Interstitial collagens are 3,000 Å long and 15 Å in diameter. The conformation and most of the properties of native collagen are determined by the triple helix domain which composes more than 95% of the molecule. This domain consists of three chains (alpha chains), each containing approximately 1,000 amino acids, wrapped in rope like fashion to form a tight, triple helix structure. The triple helix is wound in such a way that peptide bonds linking adjacent amino acids are buried within the interior of the molecule.

In native molecules the triple helix retains its resistance to attack by general proteases such as pepsin. Collagen molecules (tropocollagen) found in the extracellular matrices also contain short (e.g. about 16–25 peptide unit) non-helical extension peptides, called "telopeptides", at both the NH— and COOH— terminal ends of each alpha chain. These telopeptides are susceptible to proteolytic degradation and removal under conditions in which the triple helical body is left intact (as atelopeptide collagen).

Native collagen is generally present in connective tissue as telopeptide-containing tropocollagen molecules in side by side packed condition in the form of fibrils, with each longitudinal course composed of slightly longitudinally spaced apart molecules in end to end disposition, staggered longitudinally relative to the next successive laterally adjacent longitudinal course, thereby resulting in holes between facing end regions of successive molecules in a given longitudinal course and bounded by the staggered sides of the molecules in the parallel longitudinal courses laterally adjacent thereto.

These fibrils, e.g. of about 5 to 7 parallel courses packed together, in turn are arranged in bundles to form fibers which, along with the cells themselves, exist in the tissue in a ground substance of noncollagenous material as matrix. In bone, such holes in the staggered packing arrangement may contain mineral substances such as calcium phosphates.

In this native form, adjacent telopeptide-containing end moieties of a given molecule in a fibril are crosslinked to helical regions of adjacent molecules. The helical or central regions of the polypeptide chains or strands of a given molecule are crosslinked to each other (intramolecular crosslinks) to form a triple helix. The telopeptide and helical regions of neighboring molecules are likewise crosslinked to strands of neighboring molecules (intermolecular crosslinks), thereby forming hydrogen crosslinked or bonded and covalently crosslinked or condensed insoluble collagen. Where few, if any, stabilized reducible, crosslinks are present, the molecules in the fibril are considered soluble, i.e. the collagen is solubilized in aqueous salts, acids and bases, leaving unsolubilized the highly stabilized, crosslinked insoluble collagen.

The most common type of collagen isolated from many adult connective tissues such as skin, bone, tendon, and cornea is type I collagen. Each type I molecule is composed of two alpha 1 (I) chains and one alpha 2 (I) chain. The entire molecule is abbreviated alpha 1 $(I)_2$ alpha 2 (I).

Collagen is probably the first biomaterial ever used by man for surgical purposes. Dried intestine, predominantly composed of collagen, was used by Egyptian surgeons as a surgical suture as far back as 3750 B.C.

Numerous properties of collagen favor its use as a biomaterial; see Biomaterials in Reconstructive Surgery, Ch. **11**, Simpson, "Collagen as a biomaterial", pp. 109–117, The C. V. Mosby Co., 1983. It is absorbed at a rate that can be controlled by the degree of chemical treatment to which it is subjected. One can thus design collagen products which, on animal implantation, will be completely absorbed in a few days or months. One can chemically treat animal source collagen so that it becomes essentially totally non-absorbable while still retaining its hydrophilic character and its good tissue response.

Collagen has a high order of tensile strength and low extensibility, and can be reconstituted into membranes, sheets, tubes, sponges, or continuous length fibers. As a membrane, it is semi-permeable and a good support for cell growth. It has drug binding properties and is, for all practical purposes, immunologically inert.

The chemical and physical characteristics of collagen, its widespread distribution in many different tissues, and the ability to extract and purify and then reconstitute collagen into many physical forms would appear to make the natural polymer an ideal biomaterial. Many applications for collagen compositions have been suggested:

(A) solution form collagen applications: plasma expander, and drug delivery vehicle;

(B) gel form collagen applications: vitreous body additive, and cosmeticum;

(C) flour form collagen application: hemostatic agent;

(D) fiber form collagen applications: suture material, weaving of blood vessels, and valve prosthesis;

(E) film or membrane form collagen applications: corneal replacement, hemodialysis, artificial kidneys, wound dressing, hernia repair, and patches (aneurysm);

(F) sponge form collagen applications: wound dressing, bone-cartilage substitute, surgical tampon, and vaginal contraceptive; and

(G) tubing form collagen applications: vessel prosthesis, and reconstructive surgery of hollow organs.

However, until recently, the only clinically available collagen device was animal source suture material from intestines and from reconstituted collagen. Today there are at least two additional clinical devices composed of animal source collagen, to wit, hemostatic agents, and the Zyderm Collagen Implant (Collagen Corporation) or ZCI; see Grosh et al, J. Am. Acad. Dermatol., 13:792–798, 1985.

Pertinent prior art describes methods of chemically modifying soluble collagen by reactions with either amine or carboxyl groups on the collagen molecule. These methods render the solubilized collagen soluble at physiological pH. Collagen is generally solubilized by treatment with acids, including organic acids such as acetic acid and citric acid, and inorganic acids such as